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"SAFE AND EFFECTIVE STIMULATION OF NEURAL TISSUE"

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## SUMMARY

This work is a continuation of our program to examine the relation between stimulation-induced depression of neuronal excitability (SIDNE) during microstimulation in the feline cerebral cortex and stimulus pulse amplitude, pulse frequency, the total duration of stimulation, and the synchrony of the pulsing of many closely-spaced microelectrodes. Arrays of 7 or 16 activated iridium microelectrodes, with geometric surface areas of 1,600 to 2,400  $\mu\text{m}^2$  were implanted into the postcruciate gyri of 8 cats. At least 28 days after implanting the arrays, the microelectrodes were pulsed continuously for 7 hours. At the beginning and end of the 7-hour regimen, we recorded the responses evoked in the corticospinal tract by the intracortical stimulation. We measured the changes in the thresholds of only those responses that appear to have been generated by single corticospinal axons. SIDNE was quantified as the amount by which the electrical threshold of the unit-like responses increased during the 7 hours of stimulation. In all cases, the individual microelectrodes received the same stimulus. The pulse duration was 150  $\mu\text{s}$ /phase (cathodic first), the pulse amplitude was 26.5  $\mu\text{A}$  (4 nC/phase) and the pulse rate was 50 Hz. Pulsing 12 to 16 of the microelectrodes in a 16-electrode array induced significantly more SIDNE than when the microelectrodes were pulsed individually. This "mass-effect" was similar whether the electrodes were pulsed simultaneously or whether they were apportioned into 5 groups that were pulsed in an interleaved fashion. However, across the population of cortico-spinal units, there was considerably variability in the degree of SIDNE, even within a particular animal, and the electrical threshold of some units was affected little or not at all by the stimulation.

In another study, we have begun to examine the effects of prolonged intracortical microstimulation on the cross-correlation of unitary neuronal activity recorded with the stimulating microelectrodes. The objective of these experiments is to determine if the synaptic interconnectivity of cortical neurons is modified by the stimulation. In cat ic195, we obtained stable recordings of two neuronal units from one microelectrode, for a period of more than 1 month. Initially, the temporal pattern of their action potential were uncorrelated, but they gradually became correlated during 15 days in which the electrodes were pulsed for 7 hours per day at 26.5  $\mu\text{A}$  (4 nC/phase), at 50 Hz. The correlation persisted somewhat for 9 days after succession of stimulation, and became stronger after stimulation was resumed. These findings indicate that several hours of daily intracortical stimulation, using parameters that otherwise appear to be "safe" (no histologically-detectable tissue injury) may induce persisting changes in the inter-connectivity of neurons close to the stimulating electrodes.

# **I: The effects of simultaneous vs. interleaved pulsing on stimulation-induced changes in neuronal excitability**

## **INTRODUCTION**

Protocols for prolonged microstimulation must be designed so that they excite the requisite neuronal population, but do not induce histologically detectable tissue injury, and do not induce excessive depression of neuronal excitability. We have been investigating the relation between stimulation-induced depression of neuronal excitability (SIDNE), and several stimulus parameters (pulse amplitude, pulse frequency, and duration of stimulation). Our model system for intracortical microstimulation is the corticospinal neurons in the feline sensorimotor cortex (the pericruciate cortex). Preliminary results from this work were presented in QPRs #7 and 9.

## **METHODS**

### **Fabrication of the microelectrode arrays**

The shafts of the discrete iridium microelectrodes were made from iridium wire, 35  $\mu\text{m}$  in diameter. One end of each shaft was etched electrolytically to a cone terminating in a blunt tip with a radius of curvature of 5 to 6  $\mu\text{m}$ . A Teflon-insulated wire lead was micro-welded near the upper end of the shaft. The shafts were then insulated with 4 thin coats of Epoxylite electrode varnish, and each layer of insulation was baked using a schedule recommended by the manufacturer. The insulation was ablated from the tip of the shafts by an erbium laser. Most recently we have constructed a micro-machining station incorporating an excimer laser operating at 248 nm. The surface area of the exposed tip is determined by measurement of the double-layer capacitance while the tip is immersed in phosphate-buffered saline solution, using fast (100 Hz) cyclic voltammetry. The surface areas of these electrodes ranged from 1,600 to 2,400  $\mu\text{m}^2$ .

The individual microelectrodes are then assembled into arrays of 7 or 16, which extend 1.1 to 1.2 mm from an epoxy matrix, which is 3 mm in diameter. The electrodes occupy a cluster in which the inter-electrode distance is approximately 380  $\mu\text{m}$ . The arrays also contain 3 electrically inactive stabilizing pins, whose tracks also serve as fiducial marks for identifying the individual electrode during the subsequent histologic analysis. The microelectrodes are "activated" (a layer of high-valence iridium oxide

formed by anodic conversion) by potentiodynamic cycling between -0.8 and +0.7 volts with respect to a saturated calomel electrode, with the microelectrodes immersed in saturated sodium phosphate solution. The activation process is terminated when each microelectrode has a total charge capacity of 200 nC.

### **Surgical Procedure**

Aseptic technique is used during the surgical implantation of the microelectrode arrays. Young adult cats of either sex are anesthetized initially with Ketamine with transition to a mixture of nitrous oxide, oxygen and Halothane. The surgical procedure is carried out with the animal's head in a stereotaxic apparatus. The scalp and temporalis muscle are reflected and, using a Hall bone drill, a craniectomy is made over the left frontal cortex extending into the frontal sinus. The frontal air sinus is partly filled with bone cement.

Prior to implanting the intracortical microelectrode array, a monopolar recording electrode, and its accompanying reference electrode, are implanted in the cat's pyramidal (corticospinal) tract in order to record neuronal activity evoked by the stimulating microelectrodes. The recording electrode is fabricated from 0.25 mm Teflon-insulated stainless steel wire. The exposed area at the tip of the stainless steel electrode wire is approximately 0.1 mm<sup>2</sup>. In preparation for implantation, the wire is mounted in a sleeve-type cannula device, which is mounted in a stereotaxic assembly. A small burr hole is cut in the calvarium over the cerebellum, and a small incision is made in the dura. The cerebral cortex is stimulated as the recording electrode is guided into the pyramidal tract. When the tip of the electrode is in the tract, the inner introducer is retracted, and the recording and reference electrodes are sealed to the skull with bone cement.

In preparation for implanting the microelectrode array into the sensorimotor cortex, the percutaneous connector is mounted on the skull with stainless steel screws and methacrylate bone cement. A small flap, slightly larger than the array's superstructure matrix, is made in the dura over the postcruciate cortex, and the array of microelectrodes is inserted into the cortex at a velocity of approximately 1 m/sec,

using an axial introducer mounted on the stereotaxic frame. The array was covered with a sheet of muscle fascia, the cortex and fascia were covered with Gelfoam and the skull defect is sealed with cranioplasty.

### **Stimulation protocols**

The test stimulation protocols were conducted at 28 to 174 days after the implant surgery. During the stimulation, some of the cats were lightly anesthetized with Propofol.

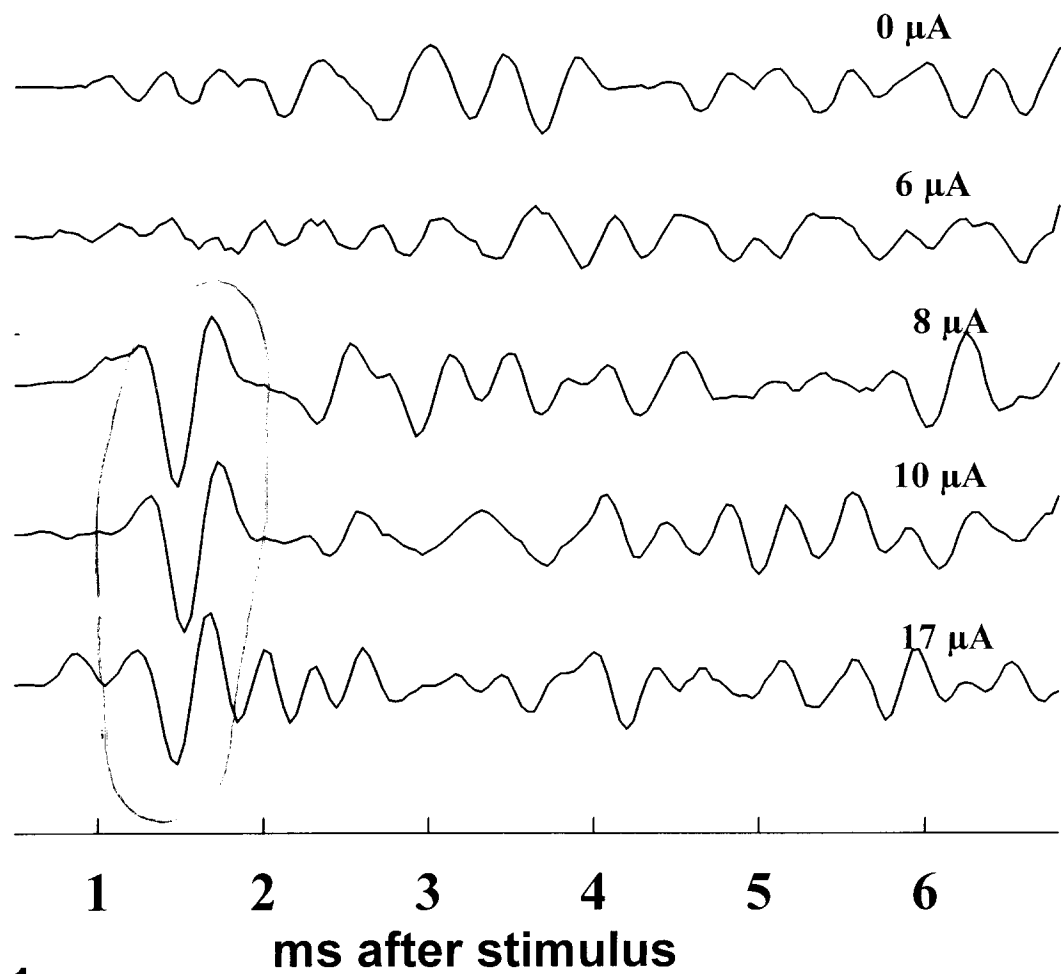
In each array, 12 to 16 (of 16) microelectrodes were pulsed continuously for 7 hours. The microelectrodes were pulsed either simultaneously or were apportioned to 5 groups that were pulsed sequentially (interleaved stimulation). The pulse duration was 150  $\mu$ s/phase (cathodic first) and the pulse amplitude was 26.5  $\mu$ A ( 4 nC/phase). The pulse rate was 50 Hz per electrode. The activated iridium microelectrodes were biased at +400 mV with respect to the implanted Ag/AgCl reference electrode.

### **Recording from the pyramidal tract**

The neuronal activity evoked in the ipsilateral pyramidal (corticospinal) tract by each of the intracortical microelectrodes was recorded before and after the sessions of continuous stimulation. Due to the sparseness of the corticospinal projection from the feline sensorimotor cortex, it was necessary to summate (average) the response to 2,048 successive stimulus pulses, in order to obtain an acceptable signal-to-noise ratio. Even after such averaging, most of the intracortical microelectrodes did not evoke a response that was large enough to be uniquely identified in successive recording sessions. When a response was present, it had the characteristics of having been generated by a single corticospinal neuron. This is due apparently to the sparseness of the corticospinal projection, and the fact that any corticospinal neurons that are excited must pass very close to the pyramidal tract recording electrodes, in order for their action potentials to be detected. Such “unitary-like” responses can be identified by their “all or none” character, and by their discrete latency after the stimulus pulse, as illustrated in Figure 1. The unit-like response is circled. In Figure 1, 2,048 consecutive responses

cat ic199 July 11, 2000. Before stimulation.

### Responses evoked from electrode 8



**Figure 1**

[h:/spw/ic/199\\_Q1.spw](h:/spw/ic/199_Q1.spw)

were summated, to obtain each trace. The abscissa is the latency after the start of the 150  $\mu$ s/phase biphasic stimulus pulse. The number near the right edge of each trace is the amplitude of the stimulus pulse. Since the response was not evoked by a stimulus pulse amplitude of 6  $\mu$ A and was present at 8  $\mu$ A, the threshold of the unit-like response was considered to be 8  $\mu$ A.

## RESULTS

Data were obtained from 8 cats with chronically-implanted intracortical arrays (Table I). The results from cats ic172-ic192 have been reported previously. In each animal, 1 to 16 of the intracortical electrodes were pulsed continuously for 7 hours, at a current of 26.5  $\mu$ A (4 nC/phase), and at a frequency of 50 Hz.

Table 1 Animals described in this report

<u>Cat</u>	<u>Date</u>	<u>Days after implant</u>	<u>Stim. mode</u>	<u># of electrodes pulsed</u>	<u># of unit-like responses</u>
ic172*	6/17/98	104	individual	1 (of 7)	1
ic175*	6/17/98	150	individual	1 (of 7)	1
ic176*	2/19/99	152	individual	1 (of 7)	1
ic176*	6/18/99	127	individual	1 (of 7)	1
ic176*	9/19/99	128	individual	1 (of 7)	1
ic178*	4/13/99	124	individual	1 (of 7)	1
ic178*	4/14/99	125	individual	1 (of 7)	1
ic178*	4/15/99	126	individual	1 (of 7)	1
ic178*	4/16/99	127	individual	1 (of 7)	1
ic192*	9/15/99	28	simultaneous	12 (of 16)	7
ic194	11/14/99	174	interleaved (5 grps)	16 (of 16)	14
ic195	4/12/99	44	interleaved (5 grps)	16 (of 16)	6
ic199	7/8/00	34	simultaneous	16 (of 16)	25
ic199	7/19/00	41	interleaved (5 groups)	15 (of 16)	19

Figure 2 shows the effects of the 7 hours of stimulation on the threshold of the unit-like responses recorded from the pyramidal tracts of the 8 cats. Each symbol represents 1 unit-like response. Symbols that would have been superimposed are

shown slightly displaced, for clarity. The latency of these responses after the stimulus pulse was less than 2 ms, so we presume that they represent corticospinal neurons that were activated directly, rather than transsynaptically, by the intracortical microstimulation. The diagonal line in each figure represents the condition in which Threshold before stimulation = Threshold after stimulation ( i.e., the 7 hours of stimulation had no effect on the response threshold). Figure 2A shows the data from cats ic172,175,176 and 178, in which only 1 of 7 microelectrodes was pulsed during each session. The 7 hours of stimulation had very little effect on the electrical threshold of the 9 unit-like activities recorded from the pyramidal tract. Figure 2B and 2C show data from cats ic192 and ic199, in which 12 (of 16) or 16 (of 16) microelectrodes were pulsed simultaneously. While the threshold of some of the units was unaffected by the 7 hours of stimulation, the threshold of most of the responses became elevated. Figure 2D,E and F show data from cats ic194,ic95 and ic199, in which 15 or 16 of the microelectrodes were pulsed in the interleaved mode, each at 50 Hz. Because of limitations of our equipment, the 15 or 16 electrodes were apportioned into 5 groups, each group containing 3 or 4 electrodes that were as far apart as possible in the array. The electrodes in each group were pulsed simultaneously at 50 Hz and the 5 groups were interleaved. With this mode of pulsing, the threshold of many of the responses became elevated during the 7 hours of stimulation.

Table II shows the raw statistics of the effect of the 7 hours of stimulation on the thresholds of the unit-like responses, in each of the three modes.

Table II

<u>Pulsing mode</u>	<u>Number of responses</u>	<u>Mean increase(<math>\mu</math>A)</u>	<u>std.Err(<math>\mu</math>A)</u>	<u>std. Dev(<math>\mu</math>A)</u>
individual	9	0.33	0.52	1.58
simultaneous	37	7.2	1.6	9.7
interleaved	46	6.7	1.25	8.2

The individual and the interleaved modes were compared, using an unpaired t-test, which showed that the magnitude of the increases in the response thresholds was

The effect of 7 hours of intracortical microstimulation  
on the threshold of pyramidal tract responses  
Individual electrodes pulsed at 26.5  $\mu\text{A}$  ( 4 nC/phase), 50 Hz  
(Response latency < 2 ms)  
Data from 4 cats

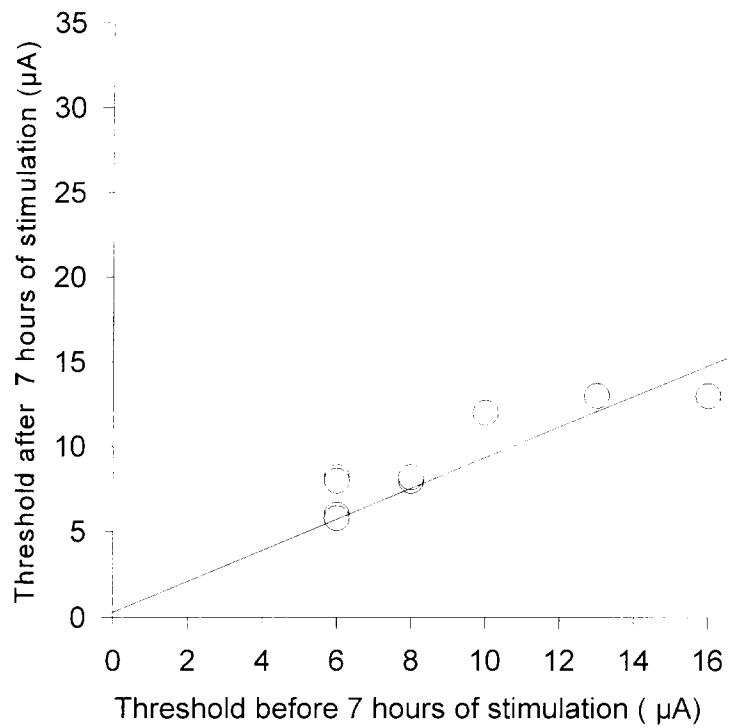


Figure 2A

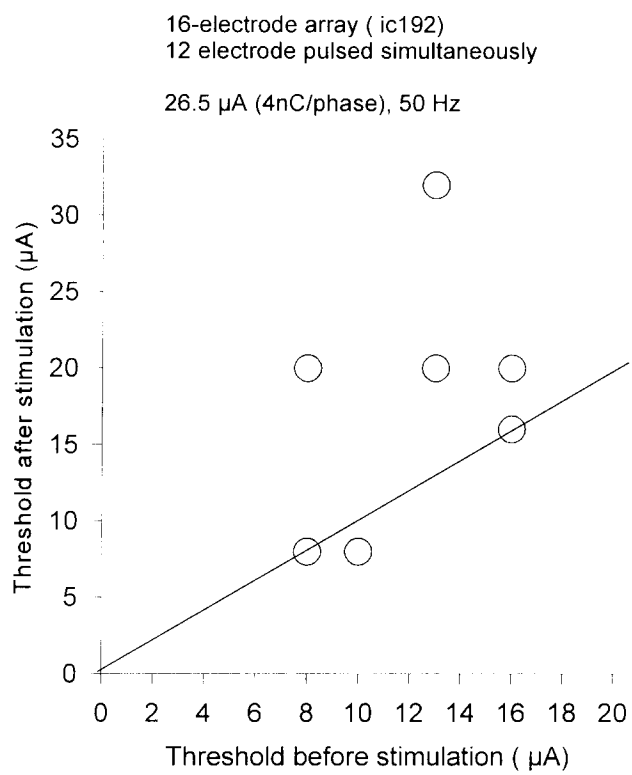


Figure 2B

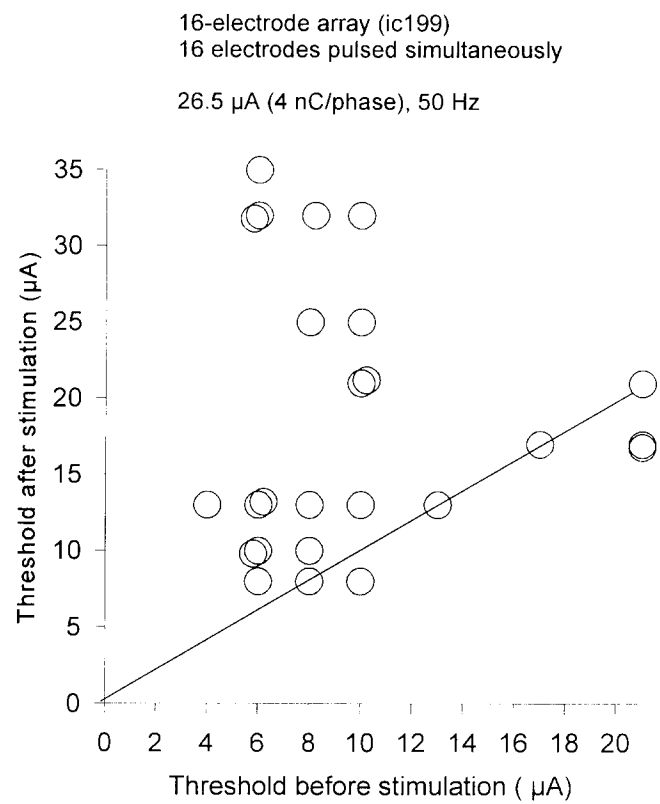


Figure 2C

16-electrode array (ic194)  
 16 electrode pulsed in interleaved mode (in 5 groups)  
 26.5  $\mu\text{A}$  ( 4 nC/ph), 50 Hz

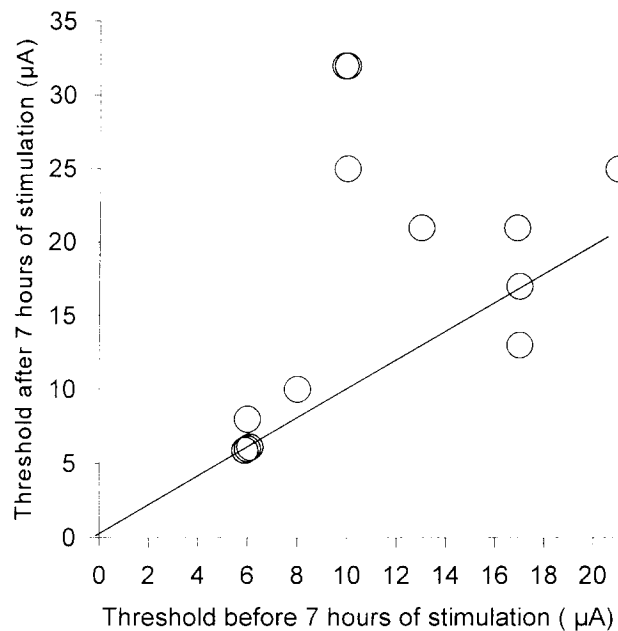
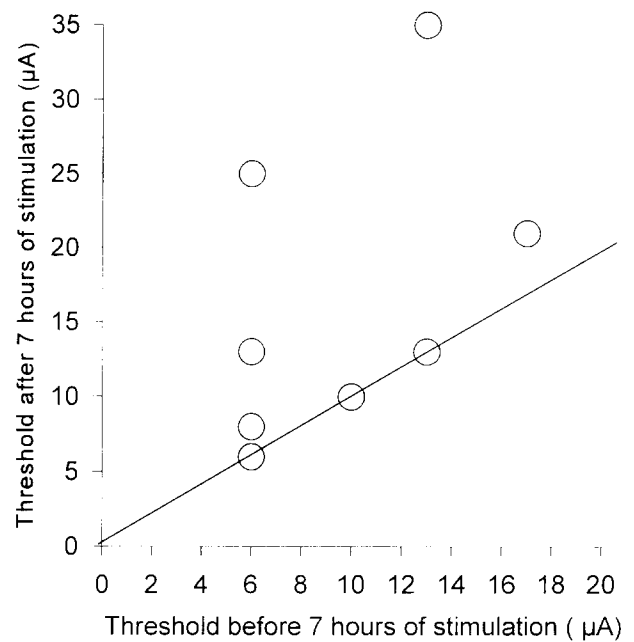


Figure 2D

16-electrode array (ic195)  
 16 electrode pulsed in interleaved mode, in 5 groups  
 26.5  $\mu\text{A}$  ( 4 nC/phase), 50 Hz



● Figure 2E

16-electrode array ( ic199)  
 15 of 16 electrode pulsed in interleaved mode, in 5 groups  
 26.5  $\mu\text{A}$  (4 nC/phase), 50 Hz

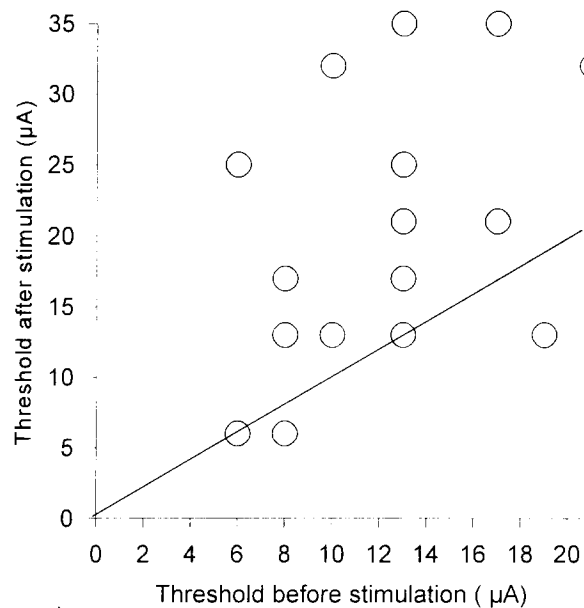


Figure 2F

significantly greater for the interleaved mode. ( $T = 2.13$ ,  $p < .02$ , one-sided test ). The simultaneously and interleaved modes were similarly compared. Although interleaved and simultaneous data were both obtained from cat ic199 ( 11 days apart, when the threshold of most of the corticospinal units had recovered to 13  $\mu\text{A}$  or less, as shown in Figure 2F) it was not possible to reliably identify individual responses across the two sessions, and the data were considered to be independent. The analysis showed that the effect of the stimulation on the response thresholds was not different for the two modes ( $T = 0.48$ ,  $p = 0.63$ ).

## DISCUSSION

The data indicate that continuous pulsing of many closely-spaced intracortical microelectrodes induces more stimulation-induced depression of neuronal excitability (SIDNE) than occurs when the electrodes are pulsed individually using the same parameters, apparently a “mass-action” phenomenon. The severity of the SIDNE is alleviated little (if at all) by interleaving the pulsing.

In the cat cochlear nucleus, we have shown that the SIDNE is considerably less when 3 closely spaced electrodes are each pulsed at 100 Hz as opposed to when 1 electrode is pulsed at 250 Hz. Also, the SIDNE is less when the adjacent electrodes are interleaved, as opposed to when they are pulsed simultaneously (McCreery et al, 1997). If SIDNE is related to the aggregate of the induced neuronal activity, then we would expect that interleaving the stimulation would reduce its severity, since simultaneous pulsing of adjacent electrodes can excite neurons that would not be excited by either electrode alone. In this context, the result from the present study of microstimulation in the feline cerebral cortex was somewhat unexpected. However, in the present study, only one level of stimulation was used (26.5  $\mu\text{A}$ , 4 nC/phase) and this may have been sufficient to activate most of the neurons within the environment of the array, in either mode of pulsing. In the cochlear nucleus, we stimulated with an amplitude-modulated signal that was intended to simulate a human voice. With this type of graded stimulation, any particular neuron would be excited more frequently ( i.e, by a greater percentage of the pulses) during simultaneous, as opposed to interleaved

pulsing, and the totality of the neuronal activity within the environment of the electrode array would be greater. The difference in the severity of the SIDNE for the individual, vs. the simultaneous or interleaved pulsing modes (Table II) indicates that the SIDNE of individual neurons may be related to the totality of the activity induced in many neurons. In this context, the benefit of interleaved pulsing may only be realized when the stimulus amplitude is low, relative to the inter-electrode distance (most neurons ordinarily would not be excited when one electrode is pulsed), when a graded stimulus is used, or when there tends to be a strong gradient in the amplitude of the stimulus across the array. In a cortical sensory prosthesis, the latter may only be possible if the dynamic range of the stimulation-evoked percept is sufficiently large.

The large variance in the severity of the SIDNE for different corticospinal neurons is yet another puzzle. The variance is large within particular animals, for both simultaneous and interleaved pulsing (Figure 2) as well as for the pooled data (Table II). The data from cat ic199 (simultaneous pulsing mode) does seem to suggest that the neurons with the lowest pre-stimulus threshold tend to experience the greatest amount of SIDNE (Figure 2C) but overall there is no significant correlation between the unit's threshold before stimulation and the severity of the SIDNE. This suggests also that there is little or no correlation between the SIDNE and the proximity of the neurons to a stimulating electrode. There is no detectable correlation between the SIDNE and the location of the electrodes in the cluster of 16 microelectrodes (center vs periphery). In this study, we only included units with post-stimulus latencies of 2 ms or less, and within this group there was no detectable correlation between the latency and the severity of the SIDNE. Thus, if there are different populations of corticospinal neurons with different degrees of susceptibility to SIDNE, they are not readily distinguished on the basis of the conduction velocity of their pyramidal tract axons.

## **II: The effects of prolonged microstimulation on the cross-correlation of the activity of cortical neurons**

### **INTRODUCTION**

It is well-established that the mature mammalian nervous system is capable of functional modifications (Merzenich and Jenkins, 1993). Structural changes in adult cat cortex following intracortical microstimulation also have been reported (Corinna and Charles, 1994). Neurons adjacent to implanted microelectrodes are stimulated simultaneously. It is important to determine if these neurons form new synaptic connections or if the strength of existing connections changes during prolonged microstimulation. If there are changes in the interconnection of the stimulated cortical neurons, this factor should be considered when we design microelectrode arrays and choose stimulation parameters.

### **METHODS**

We used a point-process cross-correlation technique to evaluate changes in the connectivity of neurons that are adjacent to the stimulating microelectrodes, and hence are stimulated simultaneously. Two adult male cats (IC194 and IC195) with chronically-implanted 16-electrode intracortical arrays were used. Intracortical microstimulation was delivered to 4 of the microelectrodes for 7 hours per day. The stimulus frequency was 50 Hz, the pulse duration was 150  $\mu$ s/phase (cathodic first) and the pulse amplitude was 26.5  $\mu$ A ( 4 nC/phase). The cats were not anesthetized during the stimulation. After each daily session of stimulation, we recorded the unitary neuronal activity from each of the pulsed electrodes. The neural activities were digitized at 25 KHz, stored on the hard disk of a PC (Pentium II), and processed offline.

Each implanted electrode can usually record actions potentials (spikes) from one or more nearby neurons. We have developed a software package which can sort (assign) the spikes to single units. The sorting algorithm is based on cluster analysis and interspike-interval analysis (Liu, et al., 1997; Liu, et al., 1999). Each spike is

represented by a set of features, i.e., the amplitudes of the spikes taken at the times when the spikes from different neurons are well separated. Then each spike is represented by a point in a multi-dimensional feature space. A hierarchical cluster analysis procedure is applied to the feature space to assign each spike to one of several candidate clusters. The set of all spikes assigned to a particular cluster is designated as a neural unit. The property of the refractory period of neurons is used to prevent clusters representing different neurons from being merged (Fee, et al., 1996). Next, the spikes from the raw data record are “classified” by assigning the feature points of each spike to the nearest cluster centroid of the clusters. Our results have shown that this sorting method agrees well with standard principal component analysis and other parametric sorting methods, and has very low computational overhead.

The point-process cross correlation can be calculated for any two units recorded by the same microelectrode or by two different microelectrodes. We designate the former as intra-channel correlation, and the latter as inter-channel correlation. Assume that the time of occurrences of the spikes of neural unit 1 are  $t(1, i)$  ( $i=1, 2, 3, \dots N1$ ), and that the time of occurrences for Unit 2 are  $t(2, j)$  ( $j=1, 2, 3, \dots N2$ ). The difference in the time of occurrences for Unit 1 and Unit 2 are  $t(1, i) - t(2, j)$  ( $i=1, 2, 3, \dots N1$  and  $j=1, 2, 3, \dots N2$ ). We then compute the correlation of the time of occurrence of the spike from the two units, where one unit's spikes occur within time  $T$  seconds preceding or following those of the other unit. We partition  $T$  into  $M$  bins, each with a width  $T/M$ . Finally we count the number of occurrences of  $t(1, i) - t(2, j)$  ( $i=1, 2, 3, \dots N1$  and  $j=1, 2, 3, \dots N2$ ) that fall into each of those bins and obtain the cross-correlation of events for Unit 1 and Unit 2 .

Any tendency for synchrony of the spike activity of the two units will appear as a peak in the cross-correlation function. Such synchrony is an indication that the two neurons represented by the two neural units receive input from a common source, or that they make an excitatory synaptic connections with one another (Perkel et al, 1967, Gerstein and Perkel, 1969) .

## RESULTS

Figure 3A-D show the cross- correlation between two units recorded from microelectrode #16 in the post-cruciate gyrus of cat ic195. These cross-correlograms have been normalized on the count in the bin with the largest number of events, which is assigned a value of 1. Figure 3A was recorded before the first day of the stimulation regimen. It shows essentially no correlation between the times of occurrence of the spikes from the two neuronal units. This microelectrodes was then pulsed for 7 hours per day, at a frequency of 50 Hz and at a pulsed amplitude of 26  $\mu$ A ( 4 nC/phase). Figure 3B shows the correlation of the two units after the 15th day of this regimen. There is now significant positive correlation in the activity of the units (a peak in the correlelogram), at a latency of approximately 1 ms. Stimulation was then suspended for 9 days, and at the end of this recovery interval, the two units were still somewhat correlated (Figure 3C). Stimulation was then resumed, for 7 hours per day, for an additional 23 days (excluding weekends). At the end of this second interval, the activity from the two neurons had become even more strongly correlated (Figure 3D). These preliminary results do indicate that several hours of daily intracortical microstimulation, using parameters that otherwise appear to be safe(no histologically-detectable tissue injury) may induce persisting changes in the inter-connectivity of neurons close to the stimulating electrodes. We have not yet seen evidence of changes in the inter-connectivity of neurons whose activity is recorded from adjacent electrodes (increased inter-channel correlation )

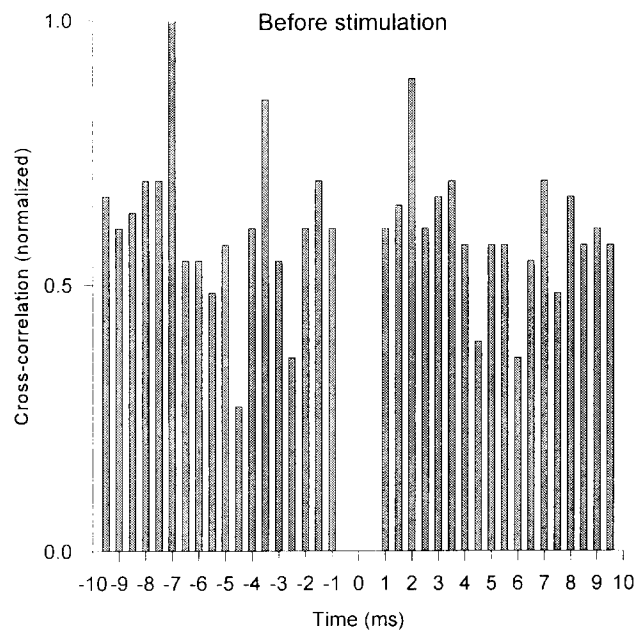


Figure 3A

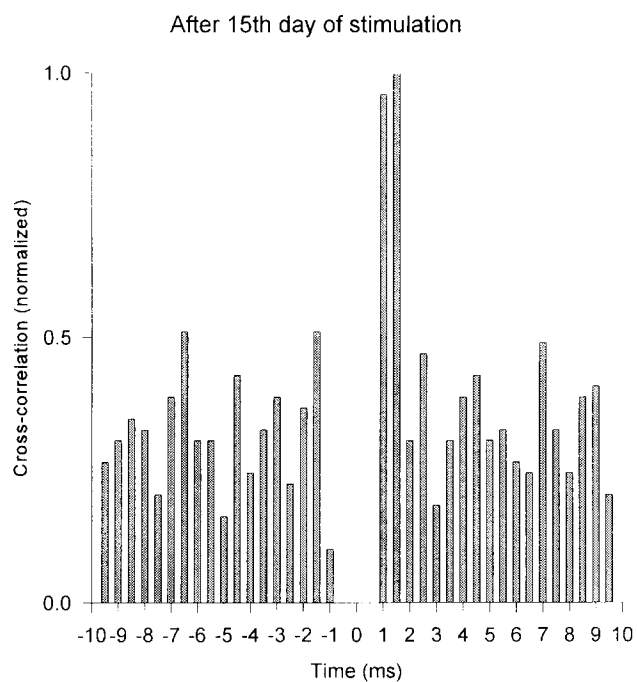


Figure 3B

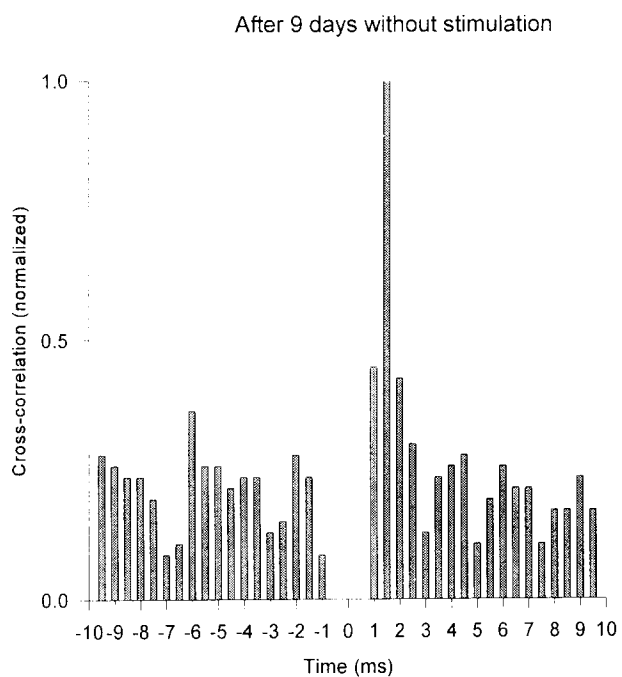


Figure 3C

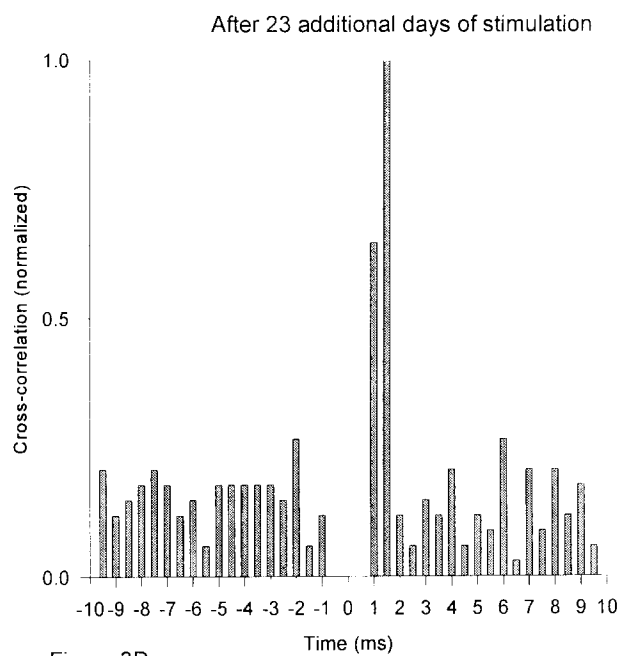


Figure 3D

## REFERENCES

- Corinna, D.S. and Charles, D.G., "Axonal sprouting accompanies functional reorganization in adult cat striate cortex," *Nature*, vol. 368, pp. 737-740, 1994.
- Fee, M.S., Mitra, P.P., and Kleinfeld, D., "Automatic sorting of multiple unit neuronal signals in the presence of anisotropic and non-Gaussian variability", *J. Neurosci. Meth.*, 69: 175-188, 1996.
- Liu, X.D., McCreery, D.B., Carter, R.R., Bullara, L.A., and Agnew, W.F., "Stability of chronically-implanted intracortical electrodes based on multi-unit recording and sorting", *Soc. Neurosci. Abstr.*, 23: 1551, 1997.
- Liu, X.D., McCreery, D.B., Carter, R.R., Bullara, L.A., Yuen, T.G.H., and Agnew, W.F., "Stability of the interface between neural tissue and chronically-implanted intracortical microelectrodes", *IEEE Trans. Rehab. Eng.*, 7 (3): 315-326, 1999.
- McCreery, D.B. T.G.H. Yuen, W.F. Agnew and L.A. Bullara, " A characterization of the effects on neuronal excitability resulting from prolonged microstimulation with chronically implanted microelectrodes," *IEEE Trans. Biomed. Eng.*, vol. 44, pp 931-939, 1997
- Merzenich, M.M. and Jenkins, W.M., "Reorganization of cortical representations of the hand following alterations of skin implants induced by nerve injury, skin island transfer and experience," *J. Hand Surgery*, vol. 6, pp. 89-104, 1993.
- Perkel, D.H, Gerstein, G.L. and Moore, G.P. "Neuronal spike trains and stochastic point processes. II: Simultaneous spike trains" *Biophys. J.* 7(4) 419-440, 1967.
- Gerstein, G.L. and Perkel, D.H. "Simultaneously recorded trains of action potentials: analysis and functional interpretation." *Science* 164 (881) 828-830, 1969